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Coleophora gryphipennella (Hübner, 1796) (Lepidoptera, Coleophoridae) on *Fragaria vesca* L. (Rosaceae), a novel host, in the coastal dunes of The Netherlands

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Abstract. During regular surveys of Lepidoptera in the coastal dune North Holland Dune Reserve, we observed larval cases and feeding traces typical for Coleophoridae on wild strawberry (*Fragaria vesca* L., Rosaceae). The spatulate or pistol shape of the cases excluded *Coleophora violacea* (Ström, 1783) and *C. potentillae* Elisha, 1885. According to the literature, *C. albicostella* (Duponchel, 1842), very rare in The Netherlands, was the only *Coleophora* species known to create spatulate cases on this host. We collected larval cases for DNA analysis on the larvae and for rearing, which revealed that none of the collected larvae belong to the Coleophoridae previously recorded feeding on this host, but a mixture of three other *Coleophora* species. We found that early instar larvae of *C. lutipennella* (Zeller, 1838) and *C. flavipennella* (Duponchel, 1843), normally feeding on oaks (*Quercus* spp., Fagaceae) only, may be found feeding on *F. vesca* in the fall. We also found that *C. gryphipennella* (Hübner, 1796), abundant on Rose (*Rosa* spp., Rosaceae) in the coastal dunes of The Netherlands, regularly feeds on *F. vesca* and rearing experiments proved that it can complete its larval stage on *F. vesca*. We therefore conclude that *Fragaria* is a new host genus for *C. gryphipennella*. After reviewing all the *C. albicostella* records from The Netherlands, we conclude that it is a very rare species, likely restricted to the southernmost provinces. None of the confirmed records are from reared specimens. The host range of *C. albicostella* in literature is possibly overestimated and may not even include *Fragaria*.

Introduction

The coastal dunes of the province North Holland constitute a region rich in Lepidoptera. The North Holland Dune Reserve, a 53 km^2 dune area roughly between the cities of Wijk aan Zee and Bergen, is a particularly rich area. Here, 1,449 species of moths (macro- and microlepidoptera) have been recorded in the Dutch national observation database "NOCTUA" (Ellis 2015a), 1,353 of which were recorded from year 2000 onwards. In total in this database approximately 2,400 different species of Lepidoptera have been recorded for The Netherlands. The North Holland Dune Reserve is protected by Natura 2000 laws and is managed by the private water company PWN. The first author is one of the volunteers who inventory Lepidoptera in this reserve. During day-time collecting, he encountered larval cases and feeding traces typical for Coleophoridae on wild strawberry (*Fragaria vesca* L., Rosaceae).

Many Coleophoridae are leaf miners as larvae, although, unlike most other leafmining groups, they can arbitrarily change their host during the larval life stage and do not stay in the leafmines continuously. The larvae build a portable case that is constructed from silk and plant tissue, usually the epidermal layers of a plant leaf (Emmet et al. 1996). The cases often have a distinctive shape and several types of cases have been designated by different authors and are included in identification keys (Hering 1951; Toll 1953; 1962; Patzak 1974; Emmet et al. 1996). However, the shape of the case can change significantly between early and later instars. Furthermore, some species shift between leaf mining to seed mining or flower or flower-bud mining, for example *Coleophora salinella* Stainton, 1859, *C. tricolor* Walsingham, 1899, and *C. bernoulliella* (Goeze, 1783) (Emmet et al. 1996). Nonetheless, most species are monophagous and the host plant is an important character for identification.

Three species of Coleophoridae were known to feed on *Fragaria* in Europe according to the literature (Hering 1957; Klimesch 1958). Two of these create lobe type cases, viz. *Coleophora violacea* (Ström, 1783) and *C. potentillae* Elisha, 1885 and can easily be recognized (Emmet et al. 1996; Ellis 2015b). The third species that had been reported to feed on *Fragaria* is *C. albicostella* (Duponchel, 1842), which creates a spatulate case, a more common case type amongst Coleophoridae. *C. albicostella* is a very rare species in The Netherlands with about a dozen registered observations or collected specimens since 1880 (Küchlein and Donner 1993; Willem Ellis, pers. comm.; RMNH and ZMA collections [Naturalis Biodiversity Center, Leiden, The Netherlands]). To find out which species we found feeding on *Fragaria* with spatulate or pistol shaped cases, we photographed, collected, and reared them and analysed their DNA.

Material and methods

Localities

The North Holland Dune Reserve stretches roughly from 52.487°N, 4.590°E to 52.682°N, 4.691°E and has an area of roughly 53 km^2. We did most of our observations in the area just south of the village Bergen aan Zee at two main localities. One is a small patch of woodland surrounded by an open dune area relatively close to the inland border of the North Holland Dune Reserve, with English Oak (*Quercus robur* L., Fagaceae) as the main tree species and *F. vesca* dominant in the herb layer, further indicated as 'L1_Oak'. The other locality is a small patch of woodland surrounded by an open dune area relatively close to the North Sea with Birch (*Betula* sp., Betulaceae) as the main tree species and again *F. vesca* dominant in the herb layer, further indicated as 'L2_Birch'. In addition, we carried out some observations in the surrounding area. An overview of all localities and dates is provided in Table 1. JvR took the photographs, with a Canon EOS 7D camera with a Canon EF 100mm f/2.8L Macro IS USM lens, except for the photographs in figures 15 and 16, which were taken with a Nikon D80 camera with a Micro-Nikkor AF 60mm f/2.8 D lens.

Rearing

On 29.x.2013 and 19.xi.2013, we collected spatulate cases on *Fragaria vesca* at the L2_Birch locality. We kept the collected larval cases in a small transparent plastic jar and put them in a garden shed to hibernate, along with small pieces of the host plant. In March 2014 we transferred the cases to fresh *Fragaria* leaves in the garden. We first observed fresh feeding traces on 11.iv.2014, the active larvae still with spatulate cases. On 05.v.2014 we observed that the most active larva had stopped feeding and that the case had moved to the petiole of the leaf it had been feeding on. The case now

Table 1. Localities of the *Coleophora* observations of this study in chronological order.

Date	GPS coordinates	Loc. name	Case type*	Host	Used for
20.v.2011	N52.638, E4.650	L1_Oak	Spatulate	*Fragaria vesca*	
24.v.2011	N52.638, E4.650	L1_Oak	Spatulate	*Fragaria vesca*	
11.x.2011	N52.644, E4.633	L2_Birch	Spatulate	*Fragaria vesca*	
20.ix.2012	N52.640, E4.653		Pistol	*Fragaria vesca*	
02.x.2012	N52.644, E4.633		Spatulate	*Fragaria vesca* and *Rosa* sp.	
19.x.2013	N52.644, E4.633	L2_Birch	Spatulate	*Fragaria vesca* and *Rosa* sp.	DNA analysis
29.x.2013	N52.638, E4.650	L1_Oak	Spatulate and pistol	*Fragaria vesca*	DNA analysis
29.x.2013	N52.639, E4.650	L1_Oak	Spatulate	*Rosa* sp.	DNA analysis
29.x.2013	N52.641, E4.656		Spatulate	*Rosa* sp.	DNA analysis
19.xi.2013	N52.643, E4.633	L2_Birch	Spatulate	*Fragaria vesca*	Rearing
07.v.2014	N52.637, E4.649	L1_Oak	Spatulate	*Fragaria vesca*	Rearing

*The case types following Emmet et al. (1996)

had a trivalved appearance. We transferred the case into a small plastic jar and kept it indoors until emergence. We found the second case lying on a leaf rather than being attached to the underside and we assumed that the larva had died. We obtained another rearing result from a still fresh spatulate case on *F. vesca* on 07.v.2014 in the dunes near Egmond (GPS coordinates 52.637°N, 4.649°E) by collecting the case together with fresh leaves that were taken indoors until emergence.

DNA barcoding

We selected ten specimens from different localities and hosts for DNA analysis, see Table 2. We pulled the larvae from their case with forceps, damaging the case as little as possible. The cases are stored as vouchers in the RMNH collection. For DNA analysis we used the mitochondrial COI-barcode gene region (Hebert et al. 2003). DNA extraction and PCR amplification followed the methods described in van Nieukerken et al. (2012). We added the collecting and sequence data of the specimens to the Barcode of Life Datasystems (BOLD; Ratnashingham and Hebert 2007), under their unique RMNH registration number as sample ID. Sufficient reference material of all potential *Coleophora* species that feed on *Fragaria* was available in BOLD and we made identifications of the larvae by examining the distance to the nearest neighbour. There was a 100% match for most samples, and also the monophyly criterion was met, when we evaluated whether our sequence fitted within a monophyletic DNA barcode species cluster.

Abbreviations

RMNH Rijks Museum voor Natuurlijke Historie collection, housed at Naturalis Biodiversity Center, Leiden, The Netherlands.

ZMA Zoölogisch Museum Amsterdam collection, housed at Naturalis Biodiversity Center, Leiden, The Netherlands.

Results

Field observations

In the spring of 2011 at locality 'L1_Oak', during an intensive search for *Tinagma perdicella* Zeller, 1839 (Douglasiidae), which flies during daytime and can be found close to *Fragaria*, JvR

noticed occupied Coleophoridae larval cases and feeding damage on *Fragaria* (Figs 1, 2). These spatulate cases at first impression had a shape similar to cases of *Coleophora albicostella*, and given the host plant, led to the assumption that this was the rare *C. albicostella*. However, rearing was unsuccessful, leaving the identifications unconfirmed. In the autumn of that same year, again spatulate cases were found, this time at locality 'L2_Birch'. At that time rearing was not attempted. It was assumed to be easier to look for larval cases in the spring and attempt to rear those, allowing the larvae to hibernate under natural conditions. However, in the spring of 2012 JvR encountered no cases and only in the next autumn, JvR observed cases again.

In the autumn of 2012 at locality 'L2_Birch' we stumbled upon a small *Rosa* sp. bush with almost each leaf occupied by a *Coleophora* case, and most leaves were eaten out. At the same locality, a few meters further, we discovered a *Fragaria vesca* plant also with typical *Coleophora* feeding traces and with cases indistinguishable from the ones on the *Rosa* sp. bush. Both even showed the small, approximately three mm long, rectangular shaped first stage cases still present on the plants (Figs 3–6). Only three cases were found on *F. vesca* and JvR decided to return in the spring to collect cases rather than to attempt to hibernate and rear these cases.

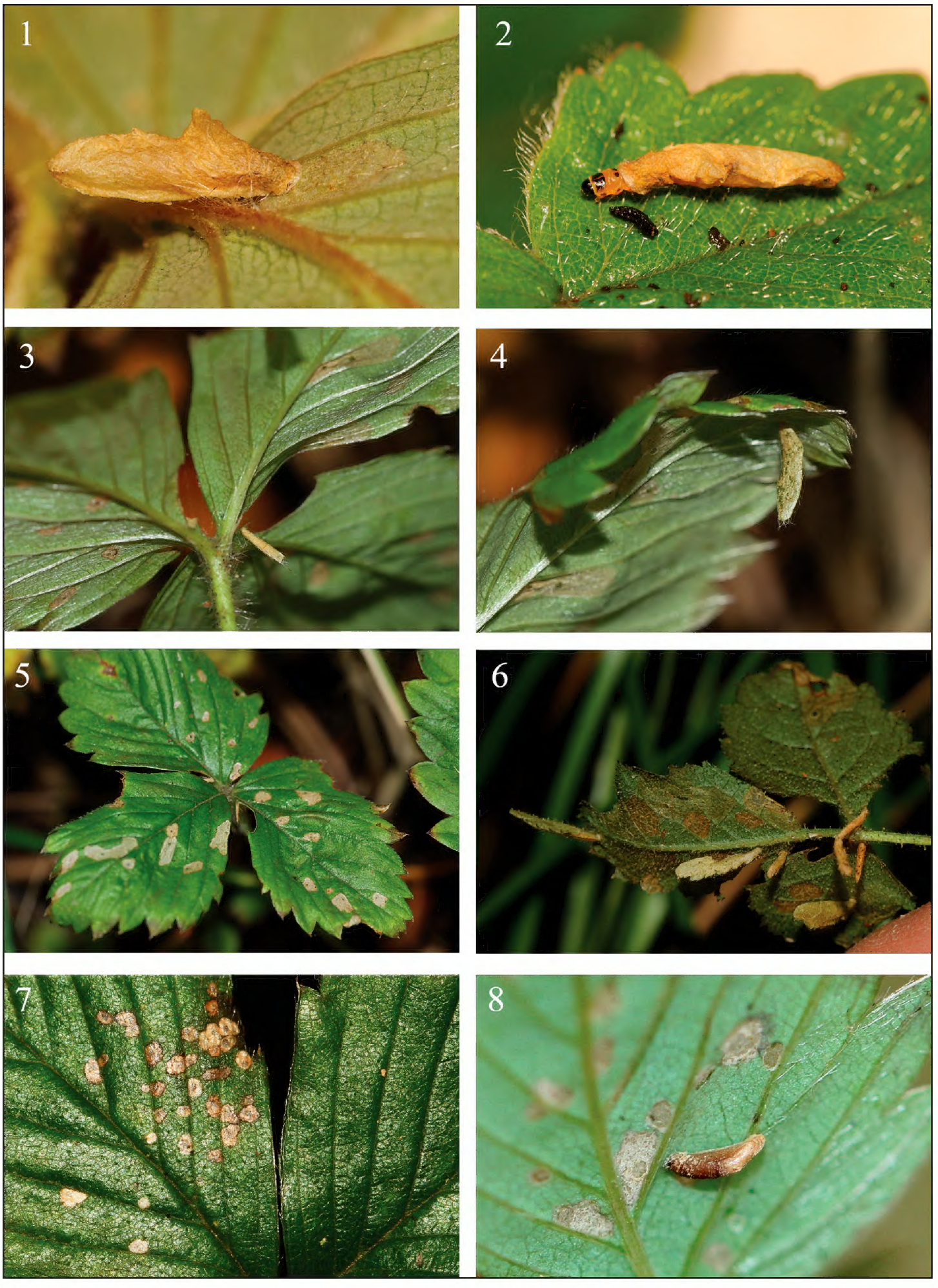
However, in the spring of 2013 again JvR found no larval cases on *Fragaria*. It took until October 2013 until finally JvR could collect cases. At this point collaboration started with CD to use DNA barcode analysis to identify the species that creates these cases on *Fragaria* and *Rosa*. To collect fresh material, we intensified the search for larval cases and this yielded two cases from *Rosa* spp. as well as from *Fragaria vesca* from different localities (Table 1). During this collecting effort, we found a second type of feeding trace and larval *Coleophora* case on *F. vesca*: tiny fleck mines created by larvae in small pistol-shaped cases (Figs 7–8) at locality 'L1_Oak'. We sequenced a total of ten larvae (see Figs 8–10) from the different localities on 19.x.2013 and 29.x.2013 (Table 2).

DNA barcode analysis

Nine out of the ten barcoded specimens yielded a DNA barcode. All identifications were unambiguous (Table 2). None of the specimens proved to be *Coleophora albicostella*. Instead, a mixture of three other species, none of which had been recorded to feed on *Fragaria*, was found: *C. gryphipennella* (Hübner, 1796), *C. lutipennella* (Zeller, 1838), and *C. flavipennella* (Duponchel, 1843). The

Table 2. Material collected for DNA barcoding and subsequent molecular identification.

Date	Loc. name	Case type	Host	RMNH number	DNA barcode identification
19.x.2013	L2_Birch	Spatulate	*Fragaria vesca*	RMNH.INS.544235	*C. gryphipennella*
29.x.2013	L1_Oak	Spatulate	*Fragaria vesca*	RMNH.INS.544234	*C. gryphipennella*
29.x.2013	L1_Oak	Spatulate	*Fragaria vesca*	RMNH.INS.544233	*C. gryphipennella*
19.x.2013	L2_Birch	Spatulate	*Rosa* sp.	RMNH.INS.558006	*C. gryphipennella*
29.x.2013	L1_Oak	Spatulate	*Rosa* sp.	RMNH.INS.544232	failed
29.x.2013	L1_Oak	Spatulate	*Rosa pimpinellifolia*	RMNH.INS.558007	*C. gryphipennella*
29.x.2013	L1_Oak	Pistol	*Fragaria vesca*	RMNH.INS.544236	*C. lutipennella*
29.x.2013	L1_Oak	Pistol	*Fragaria vesca*	RMNH.INS.544237	*C. lutipennella*
29.x.2013	L1_Oak	Pistol	*Fragaria vesca*	RMNH.INS.544239	*C. lutipennella*
29.x.2013	L1_Oak	Pistol	*Fragaria vesca*	RMNH.INS.544238	*C. flavipennella*



Figures 1–8. 1. Spatulate case found on *Fragaria* 20.v.2011. To the right of the case feeding damage can be seen. **2.** Spatulate case found on *Fragaria* 20.v.2011, with larva. **3.** First stage case on *Fragaria*, showing also the place where the leaf was cut for a second stage case, 02.x.2012. **4.** Spatulate case on *Fragaria*, 02.x.2012. **5.** *Fragaria* leaf from Figure 3 showing feeding damage, first stage case and 'leaf cut', 02.x.2012. **6.** First stage cases and spatulate cases on *Rosa*, 02.x.2012. **7.** Tiny fleck mines on *Fragaria*, 29.x.2013. **8.** Small pistol case on *Fragaria*, 29.x.2013.

larger cases, i.e. >5 mm long, were all of the spatulate type and were identified as *C. gryphipennella*. The smaller cases were pistol shaped. These were either *C. lutipennella* or *C. flavipennella*, species that normally feed on oaks (*Quercus* spp.). We did not find larger pistol shaped cases.

Rearing

We collected a few autumn cases from *Fragaria vesca* in November 2013 for rearing. They hibernated in a garden shed and began feeding on *Fragaria* again in the spring (Figs 11–12). On 21.v.2014 an adult emerged. Based on the external characters we identified it as *Coleophora gryphipennella*, which can be readily distinguished from *C. albicostella* by the lack of a white stripe along the costa (see Figs 13–14). In addition, a fresh case collected by Luc Knijnsberg on *F. vesca* on 7.v.2014 (Fig. 15) at 'L1_Oak' also successfully yielded an imago of *C. gryphipennella* on 10.vi.2014 (Fig. 16).

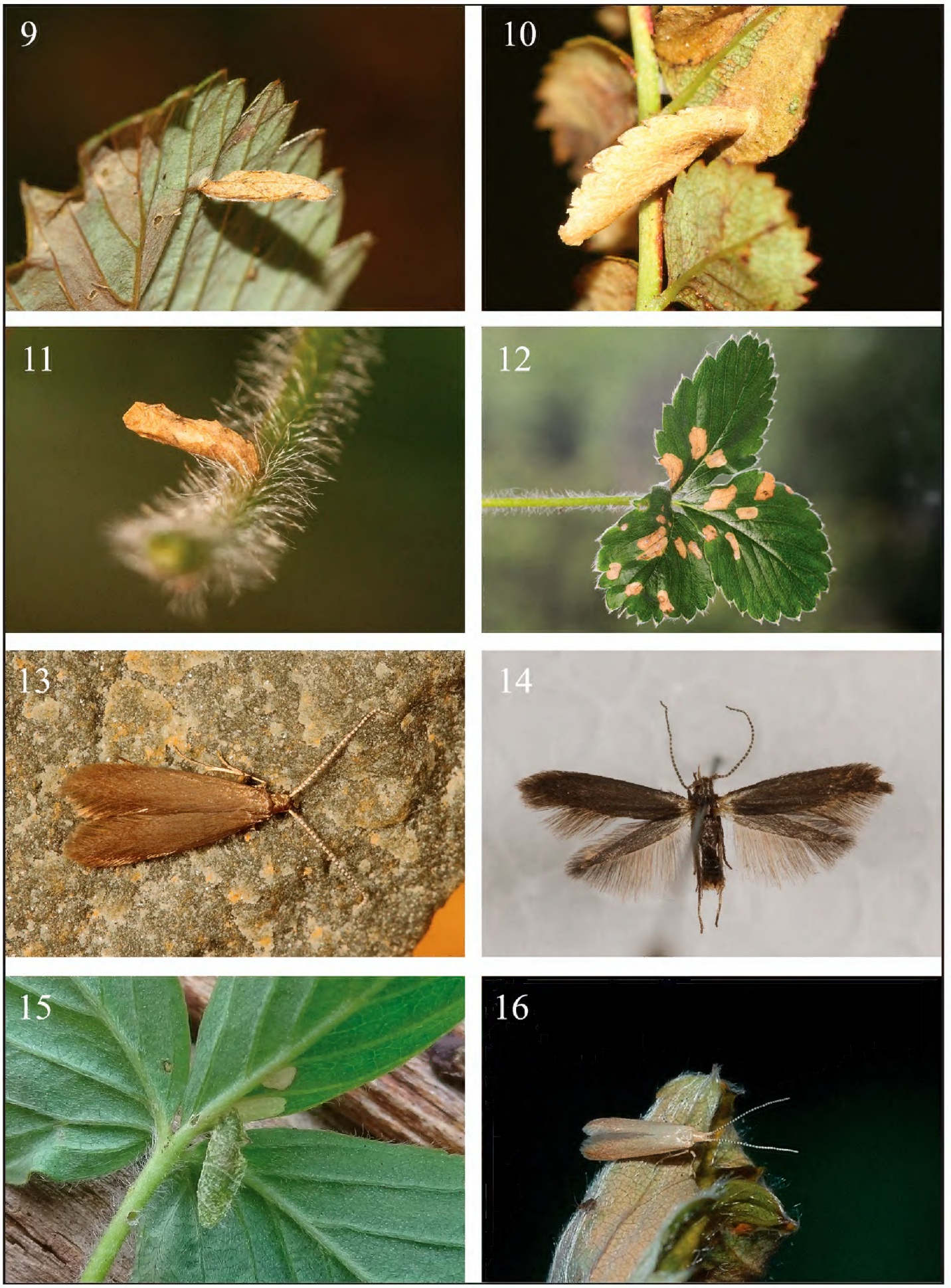
Discussion

Host range and distribution of *Coleophora albicostella*

We initially expected to have found the rare *Coleophora albicostella* in the North Holland Dune Reserve, but none of the specimens that we reared or analysed for DNA proved to be *C. albicostella*. Although this does not completely exclude the option that *C. albicostella* occurs on *Fragaria vesca* in the coastal dunes of The Netherlands, further investigation of the host records of this species made it even more unlikely. *Fragaria* spp. is first mentioned as a host plant for *C. albicostella* by Hering (1957), along with *Potentilla* spp. and *Rubus* spp. The status and source of these observations are unclear, as they are mentioned without reference to the stage (e.g. larval or adult stage) of the studied material or whether rearing was attempted. Hering (1957) does provide a drawing of the larval case, which appears as a rather general looking spatulate case that cannot be distinguished from, for example, *C. gryphipennella*. Furthermore, the case may include slight differences based on the host plant that was included in its construction. Huemer (1988) only mentions *Potentilla* spp. and occasionally also *Comarum* spp., *Filipendula* spp., *Rubus* spp., and *Sanguisorba* spp. as hosts (all Rosaceae). Rearing has been documented from *Potentilla* spp. (Bryner 2015; Richter 2015) and *Geum* spp. (Hugo van der Wolf, pers. comm., in collection). We believe that these records represent the predominant hosts. Even if *C. albicostella* may feed on the other genera, it is likely only so in areas where *Potentilla* or *Geum* may be found. In any case, also in the light of our findings here, *C. albicostella* cannot readily be reported when spatulate larval cases are encountered on *Fragaria* and should always be reared, or DNA barcoded, to efficiently identify such larvae.

Fragaria vesca as a new host for *Coleophora gryphipennella*

We demonstrated both by DNA analysis on the larvae from spatulate shaped larval cases collected on *Fragaria vesca* in the fall as well as by successfully rearing adults from larvae that have been feeding on *F. vesca* both in the fall and in the spring that *F. vesca* is a novel host for *Coleophora gryphipennella*. At least in the study area, this seems unlikely to be due to a shortage of *Rosa*, especially *Rosa pimpinellifolia* L. but also larger species such as *Rosa rubiginosa* L. are abundant and *C. gryphipennella* can be found in many localities feeding on *Rosa* throughout the habitat. The adaptation to another host plant for *C. gryphipennella* could be a local habit, only occurring in



Figures 9–16. 9. Spatulate leaf case on *Fragaria* collected for DNA analysis, 29.x.2013. **10.** Spatulate leaf case on *Rosa* collected for DNA analysis, 29.x.2013. **11.** Final, trivalved case of the reared larva after hibernation and after feeding ended on 05.v.2014. **12.** Fleck mines produced by the reared larva after hibernation and after feeding ended on 05.v.2014. **13.** Emerged imago *Coleophora gryphipennella* reared from a hibernating larva. **14.** Emerged imago *Coleophora gryphipennella* reared from a hibernating larva. **15.** Fresh green case on *Fragaria*, 07.v.2014, leg. and photograph Luc Knijnsberg. **16.** Emerged imago from the case found on 07.v.2014, photograph Luc Knijnsberg.

the dunes of the North Holland Dune Reserve. On the other hand, it could also be a more general, widespread habit that can be found throughout its distribution in Europe. More evidence to support this came from a recent find of a *C. gryphipennella* larva on *F. vesca* near Durbuy, Belgium (Steve Wullaert, pers. comm.), as confirmed by DNA analysis (RMNH.INS.30422 on BOLD). *C. gryphipennella* is distributed throughout most of Europe, Turkey, and Central and Eastern Siberia (Baldizzone et al. 2006). *C. gryphipennella* is mostly described as monophagous on *Rosa* spp. Our observations include feeding signs and larval cases on *Rosa pimpinellifolia* and *Rosa rubiginosa*. *Rubus* sp. and *Rubus corylifolius* Sm. (Rosaceae) are mentioned as incidental host plants by Hering (1957). Hering describes that in the fall the cases are somewhat pressed together sideways and are terminated with a two-sided valve and that in the spring they become more cylindrical, ending with a three-sided valve. Our observations are that after hibernation, the cases are still flat with a two-sided valve. Before pupation they turn more cylindrical with a three-sided valve.

Status of *Coleophora albicostella* and *C. gryphipennella* in The Netherlands

Our findings indicate that *Coleophora albicostella* is rare in The Netherlands, even more rare than it appeared before we started working on this manuscript, and we have had to conclude that some of the records in the national database "NOCTUA" (Ellis 2015a) involved different species. In some cases this was due to identifications based on larval cases, but sometimes also because of a confusion with the similarly named *C. albicosta* (Haworth, 1828), which feeds on *Ulex* spp. (Fabaceae). Only a handful of verified *C. albicostella* records remain, all from the southernmost part of the country, the south of the province of Limburg. Outside this area, two doubtful records are reported, one from the southwest and one from the east of the country. *C. gryphipennella* on the other hand is abundant in The Netherlands with records throughout the country in all provinces (Küchlein and Donner 1993; Muus 2015). Most records are reported from the coastal area where *Rosa pimpinellifolia* is common, which is also reported to be the main host plant (Hering 1957).

Identification of spatulate-type *Coleophora* cases on *Fragaria vesca*

Spatulate cases on *Fragaria* in the spring or the larger cases in the autumn are most likely all *Coleophora gryphipennella*, although we cannot completely exclude *C. albicostella*. It appears that the final cases in the spring are trivalved (see Fig. 11) and from this stage the adults emerge about a month later (Fig. 15). Small pistol cases (i.e. <5 mm) that may be encountered in the autumn on *Fragaria* are *C. lutipennella* or *C. flavipennella*, and can most likely be found in the vicinity of oaks (*Quercus* spp.), the common host for these species (Hering 1957). *C. lutipennella* and *C. flavipennella* are in the same species group as *C. gryphipennella* (*sensu* Emmet et al. 1996), but we find it unlikely that they can complete their larval stage without oaks. It is more likely that, when they come down from the oaks in the autumn to find a safe place to hibernate, they consume small amounts of *Fragaria* opportunistically.

Conclusions

Fragaria is a new host genus for *Coleophora gryphipennella*, which was previously only reported to feed on *Rosa*, or occasionally on *Rubus*. *C. gryphipennella* creates spatulate type cases that may be found in the fall and the spring and the species can complete its larval life on *Fragaria vesca*. Small pistol shaped cases that may be found on *Fragaria* in the fall belong to the *Quercus* feeding species *C. flavipennella* or *C. lutipennella*. The actual host range of *C. albicostella*, which was

initially known to be the only species creating spatulate cases on *Fragaria*, remains unclear, but there are no confirmed rearing records of this species from *Fragaria*.

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